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(54) Vasoactive intestinal polypeptide analogues and use thereof.

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EP-A- 241 926
EP-A- 259 891
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Description

The present invention relates to a vasoactive intestinal polypeptide (hereinafter referred abbreviately to as "VIP") analogue, namely a novel polypeptide with biological activities as in "vasoactive intestinal polypeptide", and use thereof.

The VIP analogue according to the invention can be used as an effective ingredient for various medicines, for instance an agent for curing asthma, anaphylaxis, hyperpiesia, impotence and the like, as well as a hair tonic.

The VIP is one of peptide hormones and firstly isolated and refined by Said and Mutt in the year of 1970 from a sub-fraction, when secretin was extracted from a tissue of porcine upper small intestine. In 1974, a primary amino acid structure of the VIP has been made apparent as consisting of 28 amino acids and it has been considered that it belongs to a glucagon-secretin family.

Structure of the native VIP :

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                    5                   10
His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
     15       17          20                  25
Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
     28
Leu-Asn-NH2
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It has been confirmed that the VIP presents in nervous systems in addition to the digestive canal to develop various biological activities, for instance a relatively high vasodilating-hypotensing action; atonic action on smooth muscle; accelerating action of intestinal juice secretion, pancreatic juice and bile secretions, and tear secretion; suppressing action of gastric juice secretion; accelerating action of glycogen decomposition; accelerating action of various pituitary hormone secretions; increasing action of blood flow into penis; vasodilating action of bronchus; anti-allergic action; anti-tumor action; growing action of hair and others.

Following patent literatures have been issued in Japan on the VIP, VIP analogues and use thereof.

a) Jap. Pat. No. Sho 56 128721(A),
Anti-allergic agent;
b) Jap. Pat. No. Sho 62 - 16429(A),
Acceleration of tear secretion;
c) Jap. Pat. No. Sho 62 - 116595(A),
Anti-tumor and ulcer agent;
d) Jap. Pat. No. Sho 62 - 246595(A),
Bronchodilatation agent and hypotensor;
e) Jap. Pat. No. Sho 63 - 1799892(A),
Preparation of VIP-HCl unstable to acids;
f) Jap. Pat. No. Sho 64 - 83012(A),
An agent for growing hairs;
g) Jap. Pat. No. Hei 1 - 296996(A),
Hypotensor; and
h) Jap. Pat. No. Hei 2 - 76821(A),
External preparation for curing impotence.

Among of those biological activities, the VIP has been expected as the drugs for curing an asthma and impotence, by utilizing the bronchodilitating action and the atonic action on smooth muscle of corpus cavernosum, respectively. A structural characteristic of the VIP lies in that there is an amide structure at C-terminal, which has been estimated as an indispensable matter for developing the biological activities of VIP.

For obtaining a polypeptide having an amide structure at C-terminal in accordance with conventional and widely accepted techniques which utilize a expression microorganism such as Escherichia coli, in general, it is required to separate and purify an expressed polypeptide and then to treat the polypeptide tide with use of a special C-terminal amidation enzyme. However, such an enzymatic method can not be said as that suitable for industrial scale production , since such enzyme is expensive and yield of the objective polypeptide becomes low.

Although there is no relation to the VIP in question, the inventors have found that on motilin analogues

accelerating a peristalsis of intestines, those with homoserine or homoserine-lactone at C-terminal show biological activities in same level with or higher than the native motilin, and they have proposed a process for preparing such motilin analogues with a reasonable cost and in a large amount [Jap. Pat. Appln. No. Sho 64 (1989) - 286, corresponding to a part of USSN 07/459236 and EP-03 78 078(A1), July 18, 1990].

Further, it has been reported that a chemically synthesized VIP analogue --methionine residue at 17th position of the native VIP being substituted with leucine or norleucine-- shows biological activities similar to the native VIP [said Jap. Pat. No. Sho 62 - 246595(A)]. Therefore, it has been considered that the methionine residue at 17th position has almost no influence on useful activities of the VIP.

Hitherto, it has been considered as quite difficult to provide the VIP or VIP analogues with a reasonable price and in a large amount, since according to the prior arts, there is no way other than utilizing a synthetic process therefor or an extraction method thereof from an animal tissue, and the former requires troublesome operations due to that the VIP is polypeptide consisting of 28 amino acids, and takes a relatively long period of time in its chemical synthesis and for purifying the same, and the latter is restricted on availability of the raw material and requires troublesome purification procedures.

Further, the technique utilizing so-called "Biotechnology" can not be said as --convenient method--, since it requires a special technique for the amidation at C-terminal, as referred to hereinbefore.

A main object of the invention is, therefore, to provide a VIP analogue with biological activities in same level with or higher than native VIP, by chemically synthesizing a polypeptide with a VIP-like structure, cleaving the resulting polypeptide with cyanogen bromide to prepare another polypeptide with homoserine (inclusive of homoserine-lactone) at C-terminal and VIP-like structure, and if necessary carrying out a simple chemical reaction thereon.

An additional but important object of the invention is to provide a pharmaceutical composition for curing various diseases, and more particularly asthma and impotence, which contains as an effective ingredient the VIP analogue.

The inventors have energetically studied and investigated on polypeptides obtained through a fermentation method or chemical synthesis and with a VIP-like structure. As a result, they have found that a polypeptide obtained through the cleaving step using cyanogen bromide has homoserine residue (inclusive of homoserine-lactone residue) at C-terminal and shows desired biological activities, and that an amide or alkyl amine can easily be bond to the residue in accordance with a conventional synthetic method, to establish the invention.

Therefore, according to the invention, problems in the prior arts can be dissolved by a VIP analogue shown by the general formula of

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

wherein X is a residue of amino acid other than methionine (Met); and Y is a residue of homoserine, homoserine-lactone, amidized homoserine or a residue of homoserine-lactone reacted with a primary alkyl amine having carbon atoms not exceeding 20,
to attain the main object as referred to.

The ground of that the symbol "X" at 17th position amino acid residue is stated as the residue of amino acid other than methionine (Met) lies in that if X is Met residue, the desired polypeptide having the VIP-like activities can not be obtained, since a cleavage will also occur at Met in 17th position, when a fused protein is treated with the cyanogen bromide.

The additional object as referred to can be attained by a pharmaceutical composition for curing various diseases, which contains as an effective ingredient at least one of the VIP analogues, in an effective amount.

The invention will now be further explained in more detail with reference to Manufacturing Example, Pharmacological Test Examples as well as Medicine Preparation Examples, which shall refer to drawings, wherein

Fig. 1 is a graph showing results of measurements on reluxant activity in isolated guinea pig airway smooth muscle, in accordance with Magnus method, which reluxation is caused by VIP analogues (test samples) according to the invention, and by a pure VIP (control sample) prepared by a chemical synthesis;

Fig. 2 is a graph similar to Fig. 1, excepting that test sample of VIP analogue is [L-Leu^{17}]-VIP-Hse;

Fig. 3A is a chart showing details of that a bronchus contraction induced by histamine will be atonized by adding a test sample of [L-Leu^{17}]-VIP-Hse according to the invention;

Fig. 3B is a chart similar to Fig. 3, excepting that a control sample of native VIP was added;

Figs. 4A and 4B are charts similar to Figs. 3A and 3B, excepting that concentration of the VIP analogue

and native VIP is different.

Example

(Synthesis of VIP analogues)

In the first place, a polypeptide encoding the following amino acid sequence was synthesized with use of a peptide synthesizer (Type 430A marketed by Applied Biosystems Co.).

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln-Leu-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn-Met-Ala-Ser

A purification of the synthesized polypeptide was carried out by subjecting to HPLC with use of a μ bondasphere C-18 column (19mm x 15cm) marketed by Waters Co.

The resulting purified sample (10mg) was taken and dissolved in 70% formic acid solution (30ml). After added 50mg of cyanogen bromide, the solution was kept for 24 hours at 37°C to cause a reaction. Then, distilled water (200ml) was added to the reaction mixture and lyophilized to remove the formic acid and cyanogen bromide. The resulting material was subjected again to HPLC under the following conditions, with use of the μ bondasphere C-18 column (19mm x 15cm) marketed by Waters Co. to remove the protection group and resinous materials.

Elute : Linear gradient of 15% to 50% acetonitrile in 0.1% trifluoroacetic acid (30 minutes),
Flow rate : 7.0ml/min.

Fractions in a main peak part on the HPLC were recovered and lyophilized. Through said proceedings, the synthesized polypeptide encoding said amino acid sequence has been cleaved at methionine residue part at 29th position and it has been modified into homoserine or homoserine-lactone residue at C-terminal.

The polypeptide ([L-Leu^{17}]-VIP-Hse) was further treated with an acid (for instance, in 0.1N HCl at 30°C for 3 hours) and then lyophilized, to convert homoserine residue at C-terminal into the homoserine-lactone residue in the level of 70% or more.

A part of the resulting VIP analogue was taken and checked with use of a peptide sequencer marketed by Applied Biosystems Co. to confirm that the initially synthesized polypeptide is cleaved at correct position of methionine residue at 29th position and there is the homoserine residue at C-terminal.

The polypeptide with the homoserine-lactone residue at C-terminal was collected through HPLC and lyophilized. The resulting dried powder was treated with 10% ammonia in dimethylformamide solution at room temperature for 24 hours to prepare a desired VIP analogue with homeserine-amide residue at C-terminal ([L-Leu^{17}]-VIP-Hse-NH_2).

Another type VIP analogue with an alkyl amine residue at C-terminal was prepared by reacting the polypeptide with homoserine -lactone residue at C-terminal with $CH_3(CH_2)_9NH_2$, $CH_3(CH_2)_{19}NH_2$ or the like primary alkyl amine, in dimethylsulfoxide solution.

Biological Activity Test Example 1

(Inhibition of bronchus contraction)

An inhibition of bronchus contraction was measured in accordance with a so-called "Magnus method" as disclosed in "Peptides", Vol. 6, pages 597 - 601 (1985) which uses an airway smooth muscle of guinea pig, on the VIP analogues {[L-Leu^{17}]-VIP-Hse-NH_2, [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$ and [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_{19}CH_3$}, as Test Samples and a marketed pure VIP, as Control Sample [Each of the airway smooth muscles were toned with histamine (Conc. : 10^{-5}M)].

Results are shown in Fig. 1. From the Figure, it is apparent that the VIP analogues according to the invention show the inhibition substantially equal to that of the native VIP.

Biological Activity Test Example 2

(Inhibition of bronchus contraction)

An inhibition of bronchus contraction was measured as described in the Biological Activity Test Example 1, but on the VIP analogue of [L-Leu^{17}]-VIP-Hse, as Test Sample.

Results are shown in Fig. 2.

Further, a chart showing reluxant activity of the VIP analogue and native VIP in concentration of 10^{-7}M and 10^{-8}M are given in Figs. 3A and 3B as well as Figs. 4A and 4B, respectively.

It is apparent from the results shown in the Figures, the VIP analogue according to the invention is more excellent than the native VIP.

Pharmacological Test Example 3

(Anti-allergic action)

(1) Preparation of immune serum

A rat was immunized with egg-albumin suspended in aluminum hydroxide gel in a conventional manner and a blood letting was carried out to obtain immune serum rich in IgE.

(2) Passive cutaneous anaphylaxis test (PCA reaction)

A male rat was anethtized with ether and hairs on back were sheared to intradenally inject by 0.1ml of the immune serum solution described in Item (1) and diluted to 5-folds, at both sides with a certain distance from a medial line on the back. After 72 hours, a mixed solution of egg albumin (5mg/kg), 2% Evans blue and a test compound ([L-Leu^{17}]-VIP-Hse or pure native VIP) or disodium cromoglycate was injected into a vein. After 30 minutes from an occasion of PCA reaction, the experimental animal was killed and pealed-off the skin to obtain a piece of the skin dyed with the coloring matter.

The coloring matter was extracted in accordance with the method described by Katayama ["Microbio. Immunol.", Vol. 22, No. 2, pages 89 (1978)] and an amount thereof was measured at 620nm in wave length to calculate an inhibition of the PCA reaction.

Results are shown in following Table 1.

Table 1

Dose (mg/kg)	Inhibition (%)		
	A	B	C
0.01	31	23	5
0.1	79	72	28
1.0	68	67	31

In the Table,

A : [L-Leu^{17}]-VIP-Hse;

B : Native VIP; and

C : Disodium cromoglycate.

Pharmacological Test Example 4

(Hypotensing action)

In a femoral artery of anethtized beagle dog, each of the VIP analogues as Test Samples was injected in a dose of 0.02 - 10 μ g/kg and measured an arterial pressure to prepare a chart showing a relation between the dose and change in blood pressure and check an amount of dose which shall cause a reduction of 15mmHg in blood pressure.

Following Table 2 shows an effect of the VIP analogues with a relative value, when an amount of dose of native VIP causing a reduction of 15mmHg in blood pressure shall be made as 100%.

Table 2

Native VIP	100 (%)
[L-Leu^{17}]-VIP-Hse	21 (%)
[L-Leu^{17}]-VIP-Hse-NH_2	54
[L-Leu^{17}]-VIP-$NH(CH_2)_9CH_3$	68

As apparently seen from the Table, the VIP analogues according to the invention show far excellent hypotensing action than the native VIP.

Pharmacological Test Example 5

(Accelerating action on sexual function)

After exenterating testiculus of male rats (mean body weight : about 250g) to castrate the same, testosterone (4μ g/kg) was continuously injected to each animal over a period of 16 days.

An external cream (Medicine Preparation Example 11 given later and consisting of [L-Leu^{17}]-VIP-Hse-NH_2, tannic acid and white petrolatum) was applied to a genital organ of each castrated animal (10μ g/animal as [L-Leu^{17}]-VIP-Hse-NH_2) and each experimental animal was lived in a cage with a female rat she has sexual acceptability, to check and record a number of times of copulation, over a period of 15 minutes.

As to another external cream consisting of [L-Leu^{17}]-VIP-Hse-NH_2 and white petrolatum, a test similar to the above was carried out.

Results are shown following Table 3.

Table 3

Animal	Control	Test Group A	Test Group B
No. 1	9	13	21
2	6	12	14
3	3	16	15
4	7	11	10
5	-	10	13
6	10	14	17

In the Table,

Control : Group applied a cream consisting of white petrolatum only;

Test Group A : Group applied the cream consisting of [L-Leu^{17}]-VIP-Hse-NH_2 and white petrolatum; and

Test Group B : Group applied the cream consisting of [L-Leu^{17}]-VIP-Hse-NH_2, tannic acid and white petrolatum.

The result given in the Table shows facts that the VIP analogues strengthen the sexual function, and that the tannic acid provides a rapid or immediate action in some extent.

Pharmacological Test Example 6

(Influence on growth of hair)

Hairs on back of each rabbits (mean body weight : about 2.5kg) were cut with an electric hair-clipper and the cut area was shaved. The experimental animals were classified into 6 groups (5 heads for each group). The shaved area was divided into 4 sections. A control solution (aqueous solution of saponin, which contains the saponin in a ratio of 50mg to 5ml of water) was applied on the 2 sections and a test solution selected from the followings was applied on remaining 2 sections by an amount of about 0.2ml/day for each section with an area of 9cm^2, over a period of 4 weeks.

Test Solutions

A : Aqueous solution according to Medicine Preparation Example 6 given later and containing [L-Leu^{17}]-VIP-NH$(CH_2)_9CH_3$ and saponin,

B : Aqueous solution with a similar prescription to the Solution A excepting that the VIP analogue is [L-Leu^{17}]-VIP-Hse,

C : Aqueous solution with a similar prescription to the Solution A excepting that the VIP analogue is [L-Leu^{17}]-VIP-Hse-NH_2,

D : Aqueous solution of [L-Leu^{17}]-VIP-NH$(CH_2)_9CH_3$, which contains the VIP analogue in a ratio of 5mg to water of 5ml,

E : Aqueous solution of [L-Leu^{17}]-VIP-Hse, which contains the VIP analogue in a ratio of 5mg to water of 5ml, and

F : Aqueous solution of [L-Leu^{17}]-VIP-Hse-NH_2, which contains the VIP analogue in a ratio of 5mg to water of 5ml.

On the day after the final application, an accelerating action of hair growth was evaluated under following standards for the judgement.

Standards for judgement

Score, 2 points :

Acceleration of hair growth was recognized in 5mm or more, in comparison with hairs in the sections where the control solution was applied,

Score, 1 point :

Acceleration of hair growth was recognized in less than 5mm, in comparison with hairs in the sections where the control solution was applied, and

Score, 0 (zero) point :

No acceleration of hair growth was recognized, in comparison with hairs in the sections where the control solution was applied.

Results are shown in following Table 4.

Table 4

Test Solutions	Sum of scores
A	9
B	10
C	8
D	4
E	6
F	3

It is apparent from the Table that each of the VIP analogues according to the invention shows an acceleration of hair growth, and that the coexistence of saponin increases the acceleration.

Pharmacological Test Example 7

(Inhibition of contraction in respiratory tract)

An inhibition of contraction in respiratory tract, which shall be caused by an inhalation of VIP analogues as Test compounds, was checked with use of a guinea pig model of respiratory tract contraction to be induced with an ascaris.

The experimental animals (mean body weight : about 500g) sensitized with the ascaris were classified into following 4 groups (3 heads for each group).

Control Group

Inhalant : Saline (3ml),

Test Group A

Inhalant : Saline of a dry powder obtained by Medicine Preparation Example 5 given later, which contains [L-Leu^{17}]-VIP-Hse-NH_2 of 1mg/3ml,

Test Group B

Inhalant : Saline similar to that for Test Group A, excepting that the VIP analogue is [L-Leu^{17}]-VIP-Hse, and

Test Group C

Inhalant : Saline solution similar to that for Test Group A, excepting that the VIP analogue is [L-Leu^{17}]-VIP-Hse-NH$(CH_2)_9CH_3$.

After giving the inhaltant over a period of 5 minutes to each of the experimental animals in the groups, an ascaris solution (20mg/3ml) was given over a period of 3 minutes with use of an inhaler. Measurements of a respiratory resistance and a dynamic compliance, as indexes of the contraction of respiratory tract were carried out by 7 times, namely just before the inhalation of the ascaris solution, just after the inhalation, and each period of time after 10, 20, 30, 45 and 60 minutes from the inhalation.

Influences of the inhalants for Test Groups to the respiratory resistance and dynamic compliance are given in following Tables 5 and 6 with relative values, when values at just after the inhalation of ascaris solution, and each period of time after 10, 30 and 60 minutes from the inhalation, on the Control Group shall be made as 100%.

Table 5 (Respiratory Resistance)

	Control Group	Test Group A	Test Group B	Test Group C
Just after inhalation	100%	62%	59%	67%
After 10min.	100%	65%	53%	72%
After 30min.	100%	72%	69%	80%
After 60min.	100%	81%	78%	86%

Table 6 (Dynamic Compliance)

	Control Group	Test Group A	Test Group B	Test Group C
Just after inhalation	100%	135%	140%	132%
After 10min.	100%	134%	141%	128%
After 30min.	100%	136%	139%	123%
After 60min.	100%	131%	138%	115%

Medicine Preparation Example 1

A solution of the VIP analogue ([L-Leu^{17}]-VIP-Hse) in refined water was aseptically charged into vials, so that each vial contains the VIP analogue by 1mg. After lyophilized, the vial was sealed to obtain a dry powdery medicine. The powdery medicine is dissolved in saline or the like for injection purpose, when it shall be used.

For stabilizing the VIP analogue, a human serum albumin or the like can be used.

Medicine Preparation Examples 2 to 4

Powdery medicines were prepared as described in Medicine Preparation Example 1, excepting that one of the following VIP analogues was employed in lieu of [L-Leu^{17}]-VIP-Hse.

a) [L-Leu^{17}]-VIP-Hse-NH_2,

b) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$, and

c) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_{19}CH_3$.

Each of the powdery medicine is dissolved in saline or the like for the injection purpose, when it shall be used. For stabilizing the VIP analogue, a human serum albumin or the like can be used.

Medicine Preparation Example 5

In an aqueous solution (5ml) consisting of [L-Leu^{17}]-VIP-Hse-NH_2 (3mg) and refined water, sodium glycocholate (30mg) and mannitol (100mg) were added to dissolve the same. The solution was aseptically charged into a vial. After lyophilized, the vial was sealed to obtain a dry powdery medicine. The powdery medicine is dissolved in refined water and charged into an atomizer or nebulizer to splay the solution into the nasal fossa, when it shall be used.

Medicine Preparation Example 6

In an aqueous solution (5ml) consisting of [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$ (5mg) and refined water, saponin (50mg) was added to dissolve the same. The solution can be dosed to the nasal fossa through an application or spraying.

Medicine Preparation Example 7

A hydrogel type medicine was prepared in a conventional manner and with a following prescription. The medicine was charged into an aluminum tube for packing such type medicine.

[L-Leu^{17}]-VIP-Hse-NH_2	300 (mg)
Hydroxypropylmethylcellulose	100
Tween or polysorbate (Note: Trademark) 60	100
Gelatin	500
70% Aqueous solution of sorbitol	2000
Citric acid	100
Disodium hydrogenphosphate	300
Sodium chloride	500
Benzalkonium chloride	20
Refined water	remainder
Total	100 (g)

Medicine Preparation Example 8

To prepare a granular preparation of 1g in dose for each time, [L-Leu^{17}]-VIP-Hse-NH_2 (10mg) and sucrose palmitate (200mg) were mixed. To the mixture, lactose, starch and hydroxypropylcellulose were added in a suitable amount to prepare granules in a conventional manner. Then, an enteric coating was applied thereto with use of hydroxypropylmethylcellulose (P).

Medicine Preparation Example 9

Tablets were prepared in a conventional manner and with a following prescription.

[L-Leu^{17}]-VIP-Hse	10 (mg)
Sodium laurylsulfate	20
Carboxymethylcellulose (Ca)	7
Crystalline cellulose	2
Magnesium stearate	7
Lactose	remainder
Total	200 (mg)/tablet

Medicine Preparation Example 10

A suppositories were prepared in a conventional manner and with a following prescription.

[L-Leu^{17}]-VIP-Hse	20 (mg)
Tannic acid	30
Ichthamol	300
Cacao butter	remainder
Total	1000 (mg)/piece

Medicine Preparation Example 11

An external cream or ointment was prepared in a conventional manner and with a following prescription.

[L-Leu^{17}]-VIP-Hse-NH_2	100 (mg)
Tannic acid	100
White petrolatum	remainder
Total	100 (g)

Medicine Preparation Example 12

An ophthalmic solution was prepared in a conventional manner and with a following prescription.

[L-Leu^{17}]-VIP-Hse	1000 (mg)
Sodium dihydrogenphosphate (anhydride)	560
Disodium hydrogenphosphate (anhydride)	370
Sodium chloride	370
Benzalkonium chloride solution (diluted to 1/50000)	remainder
Total	100 (ml)

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. A vasoactive intestinal polypeptide (VIP) analogue shown by the formula of

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

wherein X is a residue of amino acid other than methionine (Met); and Y is a residue of homoserine, homoserine-lactone, amidized homoserine or a residue of homoserine-lactone reacted with a primary alkyl amine having carbon atoms not exceeding 20.

2. A vasoactive intestinal polypeptide (VIP) analogue as claimed in Claim 1, wherein said analogue is selected from the group consisting of
   a) [L-Leu^{17}]-VIP-Hse,
   b) [L-Leu^{17}]-VIP-Hse- NH_2,
   c) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$, and
   d) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_{19}CH_3$.

3. A pharmaceutical composition comprising in an effective amount a vasoactive intestinal polypeptide (VIP) analogue shown by the formula of

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

wherein X is a residue of amino acid other than methionine (Met); and Y is a residue of homoserine,

homoserine-lactone, amidized homoserine or a residue of homoserine-lactone reacted with a primary alkyl amine having carbon atoms not exceeding 20, and a pharmacologically acceptable carrier.

4. A pharmaceutical composition as claimed in Claim 3 for curing asthma, anaphylaxis, hypertension and impotence.

5. A pharmaceutical composition as claimed in Claim 3 for accelerating growth of hair.

6. A pharmaceutical composition as claimed in Claim 3 for accelerating a secretion in the lachrymal gland.

Claims for the following Contracting States : ES, GR

1. Process for the preparation of vasoactive intestinal polypeptide (VIP) analogues, useful for the treatment of asthma, anaphylaxis, hypertension and impotence, as well as for accelerating growth of hair and the secretion in the lachrymal gland, of formula:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

wherein X is a residue of amino acid other than methionine (Met); and Y is a residue of homoserine, homoserine-lactone, amidized homoserine or a residue of homoserine-lactone reacted with a primary alkyl amine having carbon atoms not exceeding 20;
said process being characterized by comprising:
a) chemically synthesizing a polypeptide with a VIP-like structure, corresponding to the following amino acid sequence:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn-Met-Ala-Ser

where X has the aforesaid meaning, using a peptide synthesizer;
b) cleaving the polypeptide obtained in step (a), previously purified at 29th position, corresponding to the residue of the methionine, with a cyanogen bromide, for obtaining another polypeptide with homoserine or homoserine-lactone at C-terminal and the residue of the VIP-like structure, of formula:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn-Y_o

where Y_o represents a residue of homoserine, which can be transformed into a residue of homoserine-lactone, by treatment of the thus obtained peptide with an inorganic acid;
c) facultatively reacting the peptide obtained in step (b), where the C-terminal is a residue of homoserine-lactone, either with ammonia, for obtaining the corresponding peptide where the C-terminal is a residue of homoserine-amide, or with an alkyl amine having carbon atoms not exceeding 20, for obtaining the corresponding peptide,where the C-terminal corresponds to the residue of homoserine linked to said alkyl amine.

2. Process according to claim 1, characterized by the fact that the thus obtained polypeptide is selected from the following ones:
a) [L-Leu^{17}]-VIP-Hse,
b) [L-Leu^{17}]-VIP-Hse-NH_2,

c) [L-Leu^{17}]-VIP-Hse-NH$(CH_2)_9CH_3$, and
d) [L-Leu^{17}]-VIP-Hse-NH$(CH_2)_{19}CH_3$.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Analogon des vasoaktiven intestinalen Polypeptids (VIP), mit der Formel

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-Y

worin X einen Rest einer von Methionin (Met) verschiedenen Aminosäure bedeutet; und Y einen Rest von Homoserin, Homoserinlacton, amidisiertem Homoserin oder einen Rest von Homoserinlacton, umgesetzt mit einem primären Alkylamin mit einer 20 nicht übersteigenden Anzahl an Kohlenstoffatomen, bedeutet.

2. Analogon des vasoaktiven intestinalen Polypeptids (VIP) gemäß Anspruch 1, wobei das Analogon ausgewählt ist aus der Gruppe
   a) [L-Leu^{17}]-VIP-Hse,
   b) [L-Leu^{17}]-VIP-Hse-NH_2,
   c) [L-Leu^{17}]-VIP-Hse-NH$(CH_2)_9CH_3$ und
   d) [L-Leu^{17}]-ViP-Hse-NH$(CH_2)_{19}CH_3$.

3. Arzneimittel, umfassend eine wirksame Menge eines Analogons des vasoaktiven intestinalen Polypeptids (VIP) der Formel

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-Y

worin X einen Rest einer von Methionin (Met) verschiedenen Aminosäure bedeutet; und Y einen Rest von Homoserin, Homoserinlacton, amidisiertem Homoserin oder einen Rest von Homoserinlacton, umgesetzt mit einem primären Alkylamin mit einer 20 nicht übersteigenden Anzahl an Kohlenstoffatomen, bedeutet und einen pharmakologisch verträglichen Träger.

4. Arzneimittel nach Anspruch 3 zur Behandlung von Asthma, Anaphylaxie, Bluthochdruck und Impotenz.

5. Arzneimittel nach Anspruch 3 zur Beschleunigung des Haarwuchses.

6. Arzneimittel nach Anspruch 3 zur Sekretionsbeschleunigung in der Tränendrüse.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung von Analoga des vasoaktiven intestinalen Polypeptids (VIP) zur Behandlung von Asthma, Anaphylaxie, Bluthochdruck und Impotenz sowie zur Beschleunigung des Haarwuchses und der Sekretion in der Tränendrüse, der Formel

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-Y

worin X einen Rest einer von Methionin (Met) verschiedenen Aminosäure bedeutet; und Y einen Rest von Homoserin, Homoserinlacton, amidisiertem Homoserin oder einen Rest von Homoserinlacton, umgesetzt mit einem primären Alkylamin mit einer 20 nicht übersteigenden Anzahl an Kohlenstoffatomen, bedeutet, wobei das Verfahren umfaßt:

a) chemische Synthese eines Polypeptids mit VIP-ähnlicher Struktur, entsprechend nachstehender Aminosäuresequenz:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-Met-Ala-Ser

worin X die vorstehende Bedeutung aufweist unter Verwendung einer Peptidsynthesevorrichtung;
b) Spalten des in Schritt (a) erhaltenen, vorher gereinigten Polypeptids in der 29. Stellung entsprechend dem Methioninrest mit Bromcyan unter Erhalt eines anderen Polypeptids mit Homoserin oder Homoserinlacton als C-endständige Gruppe und dem Rest der VIP-ähnlichen Struktur der Formel:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-X-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-Y_o

worin Y_o einen Rest von Homoserin wiedergibt, der in einen Rest von Homoserinlacton umgewandelt werden kann durch Behandlung des so erhaltenen Peptids mit einer anorganischen Säure;
c) Umsetzen des in Schritt (b) erhaltenen Peptids, wobei die C-endständige Gruppe ein Rest von Homoserinlacton ist, entweder mit Ammoniak unter Erhalt des entsprechenden Peptids, worin die C-endständige Gruppe ein Rest von Homoserinamid ist oder mit einem Alkylamin, das eine Anzahl an Kohlenstoffatomen aufweist, die 20 nicht übersteigt, unter Erhalt des entsprechenden Peptids, worin die C-endständige Gruppe dem an das Alkylamin gebundenen Homoserinrest entspricht.

2. Verfahren nach Anspruch 1, **dadurch gekennzeichnet,** daß das so erhaltene Polypeptid ausgewählt ist aus den nachstehenden:
   a) [L-Leu^{17}]-VIP-Hse,
   b) [L-Leu^{17}]-VIP-Hse-NH_2,
   c) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$ und
   d) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_{19}CH_3$.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Analogue du polypeptide intestinal vasoactif (VIP), représenté par la formule :

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn- Y

dans laquelle :
- X représente un résidu d'acide aminé autre que méthionine (Met) ; et
- Y représente un résidu d'homosérine, d'homosérine-lactone, d'homosérine amidée ou un résidu d'homosérine-lactone ayant réagi avec une alkyl amine primaire ayant un nombre d'atomes de carbone ne dépassant pas 20.

2. Analogue du polypeptide intestinal vasoactif (VIP) selon la revendication 1, dans lequel ledit analogue est choisi dans le groupe constitué par :
   a) [L-Leu^{17}]-VIP-Hse,
   b) [L-Leu^{17}]-VIP-Hse-NH_2,

c) [L-Leu^{17}]-VIP-Hse-NH$(CH_2)_9CH_3$, et
d) [L-Leu^{17})-VIP-Hse-NH$(CH_2)_{19}CH_3$.

3. Composition pharmaceutique comprenant, dans une quantité efficace, un analogue du polypeptide intestinal vasoactif (VIP), représenté par la formule :

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

dans laquelle :
- X représente un résidu d'acide aminé autre que méthionine (Met) ; et
- Y représente un résidu d'homosérine, d'homosérine-lactone, d'homosérine amidée ou un résidu d'homosérine-lactone ayant réagi avec une alkyl amine primaire ayant un nombre d'atomes de carbone ne dépassant pas 20,

et un support pharmacologiquement acceptable.

4. Composition pharmaceutique selon la revendication 3, pour le traitement de l'asthme, de l'anaphylaxie, de l'hypertension et de l'impuissance.

5. Composition pharmaceutique selon la revendication 3, pour l'accélération de la pousse des cheveux.

6. Composition pharmaceutique selon la revendication 3, pour l'accélération de la sécrétion des glandes lacrymales.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation d'analogues du polypeptide intestinal vasoactif (VIP), utiles pour le traitement de l'asthme, de l'anaphylaxie, de l'hypertension et de l'impuissance, ainsi que pour l'accélération de la pousse des cheveux et de la sécrétion des glandes lacrymales, représentés par la formule:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn- Y

dans laquelle :
- X représente un résidu d'acide aminé autre que méthionine (Met) ; et
- Y représente un résidu d'homosérine, d'homosérine-lactone, d'homosérine amidée ou un résidu d'homosérine-lactone ayant réagi avec une alkyl amine primaire ayant un nombre d'atomes de carbone ne dépassant pas 20 ;

ledit procédé étant caractérisé par le fait qu'il comprend :
a) la synthèse chimique d'un polypeptide ayant une structure analogue au VIP, correspondant à la séquence d'acides aminés suivante :

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-
Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-
Leu-Asn-Met-Ala-Ser

dans laquelle X a la signification mentionnée ci-dessus, à l'aide d'un dispositif de synthèse peptidique ;
b) le clivage du polypeptide obtenu à l'étape (a), préalablement purifié,au niveau de la 29ème position, correspondant au résidu de la méthionine, par le bromure de cyanogène, afin d'obtenir un autre polypeptide ayant une homosérine ou une homosérine-lactone à l'extrémité C-terminale et le résidu de

structure analogue au VIP, représenté par la formule :

$$\text{His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln- X -Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu- Asn-}Y_0$$

dans laquelle Y_0 représente un résidu d'homosérine, qui peut être transformé en un résidu d'homosérine-lactone, par traitement du peptide ainsi obtenu par un acide minéral ;
c) la réaction du peptide obtenu à l'étape (b), dans lequel l'extrémité C-terminale est un résidu d'homosérine-lactone, soit avec de l'ammoniaque, pour obtenir le peptide correspondant dans lequel l'extrémité C-terminale est un résidu d'homosérine-amide ; soit avec une alkyl amine ayant un nombre d'atomes de carbone ne dépassant pas 20, pour obtenir le peptide correspondant, dans lequel l'extrémité C-terminale correspond au résidu d'homosérine lié à ladite alkyl amine.

2. Procédé selon la revendication 1, caractérisé par le fait que le polypeptide ainsi obtenu est choisi parmi les polypeptides suivants :
   a) [L-Leu^{17}]-VIP-Hse,
   b) [L-Leu^{17}]-VIP-Hse-NH_2,
   c) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_9CH_3$, et
   d) [L-Leu^{17}]-VIP-Hse-$NH(CH_2)_{19}CH_3$.

FIG. 1

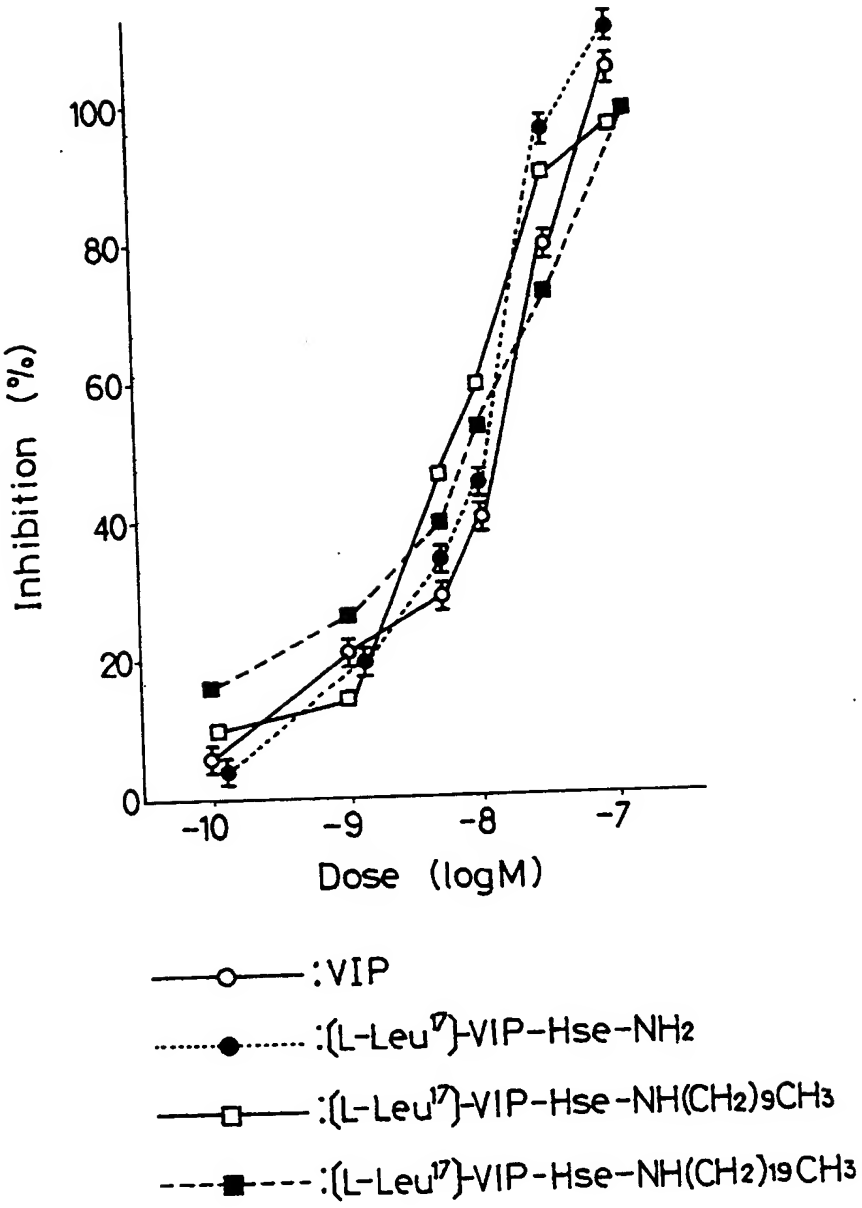

Inhibition (%)
100
80
60
40
20
0
-10
-9
-8
-7
Dose (logM)
:VIP
:(L-Leu17)-VIP-Hse-NH2
:(L-Leu17)-VIP-Hse-NH(CH2)9CH3
:(L-Leu17)-VIP-Hse-NH(CH2)19CH3

FIG. 2

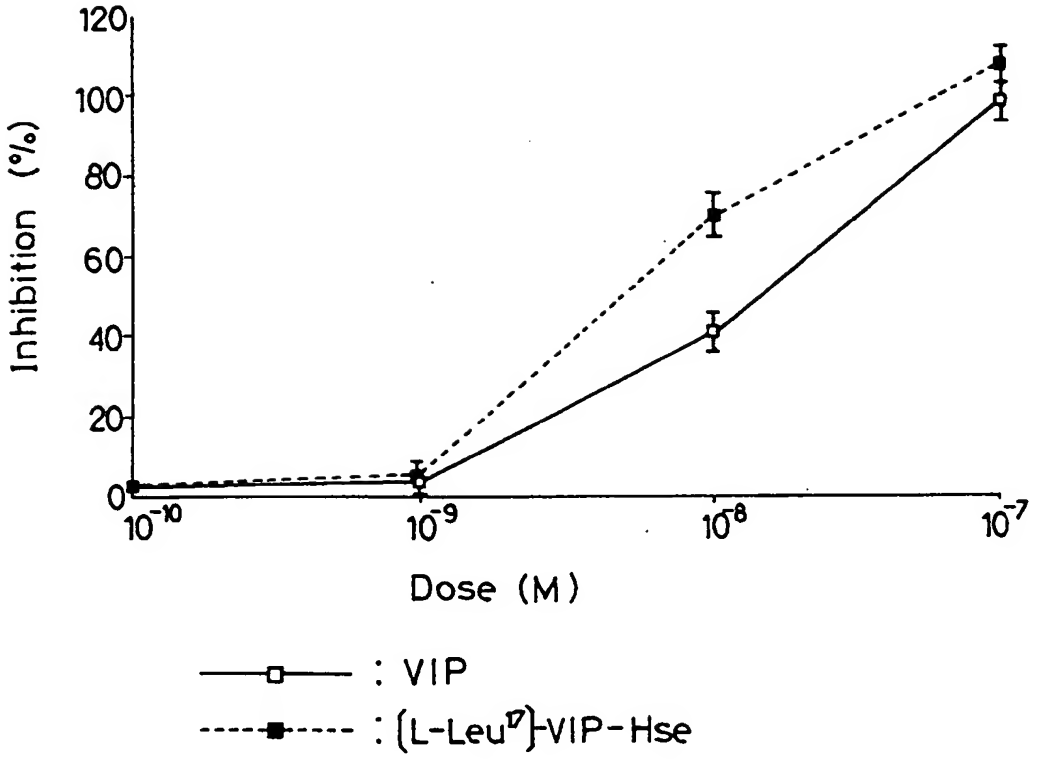
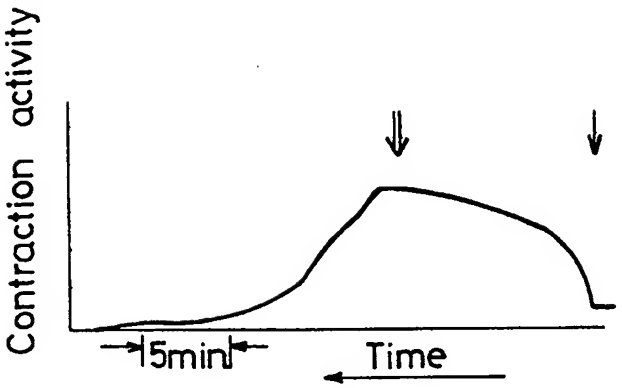

120
100
80
60
40
20
0
Inhibition (%)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Dose (M)
: VIP
: [L-Leu^{17}]-VIP-Hse

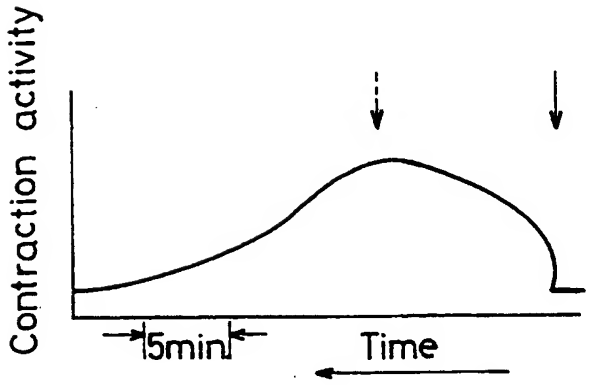
FIG. 3A



↓ : Add Histamine (10^{-5}M)

⇓ : Add [L-Leu^{17}]-VIP-Hse (10^{-7}M)

FIG. 3B



↓ : Add Histamine (10^{-5}M)

⇣ : Add native VIP (10^{-7}M)

FIG. 4A

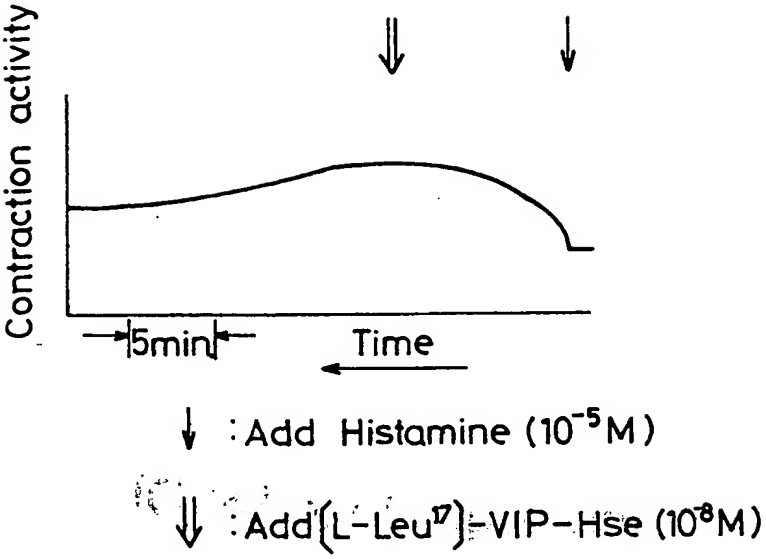
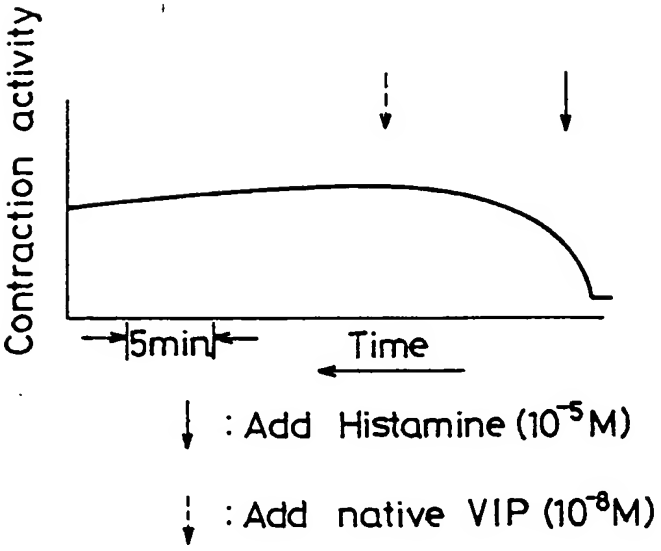

Contraction activity
5min
Time
: Add Histamine (10^{-5}M)
: Add (L-Leu^{17})-VIP-Hse (10^{-8}M)

FIG. 4B


Contraction activity
5min
Time
: Add Histamine (10^{-5}M)
: Add native VIP (10^{-8}M)